Conjugation of peptide vaccine directly to TLR agonists generates enhanced immune responses and tumor therapy

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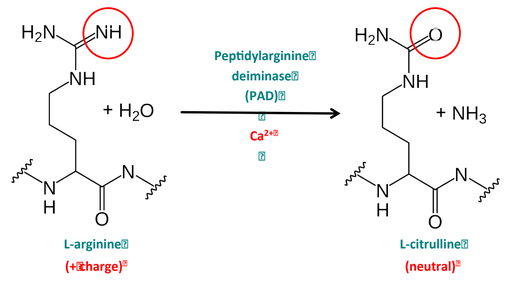
INTRODUCTION

- Peptide vaccines require adjuvants for efficient T cell stimulation and TLR ligands have been shown to have such adjuvant properties
- Mixtures of peptide with TLR agonists have been shown to be efficient for induction of CD4 and CD8 T cell responses however linkage of the peptide directly to the TLR ligand have been shown to enhance responses(1).
- Amplivant Technology developed by ISA Pharmaceuticals makes use of a synthetic TLR1/2 agonist that can be chemically coupled to a synthetic long peptide (SLP).
- Amplivant linked SLPs allow better processing and presentation by dendritic cells for induction of both CD8 and CD4 T cell responses(2).
- CD4 T cells are potent effectors but CD4 responses to self antigens are often attenuated.
- Cellular stress induces autophagy which leads to modification of proteins recognised by the immune system (4). One such modification is citrullination (cit).
- In the absence of inflammation, immunity is regulated, whereas in its presence CD4 responses to modified self-antigens are stimulated (3).
- Cancer cells citrullinate proteins (5). Citrullinated proteins in cancer cells include ubiquitous cytoskeletal protein Vimentin and glycolytic enzyme α-Enolase.
- Stressful conditions in tumour microenvironment leads to presentation of modified peptides on MHC class II which is a target for CD4 T cells. We have shown that these can be harnessed for tumour therapy (6).
- In this study we examine the effects of direct peptide conjugation to TLR1/2 and TLR9 agonists on the stimulation of citrullinated peptide specific CD4 T cells.



Citrullination. A modification that occurs within stressed cells. Peptidylarginine deiminase (PADs) enzymes are activated and convert arginine to citrulline by altering the positively charge aldimine group (=NH) group of arginine to the neutrally charged ketone group (=O) of citrulline.

Citrullinated peptides mixed with TLR9 agonist stimulate Th1 responses restricted through HLA-DR4 and HLA-DP4 which mediate tumour therapy

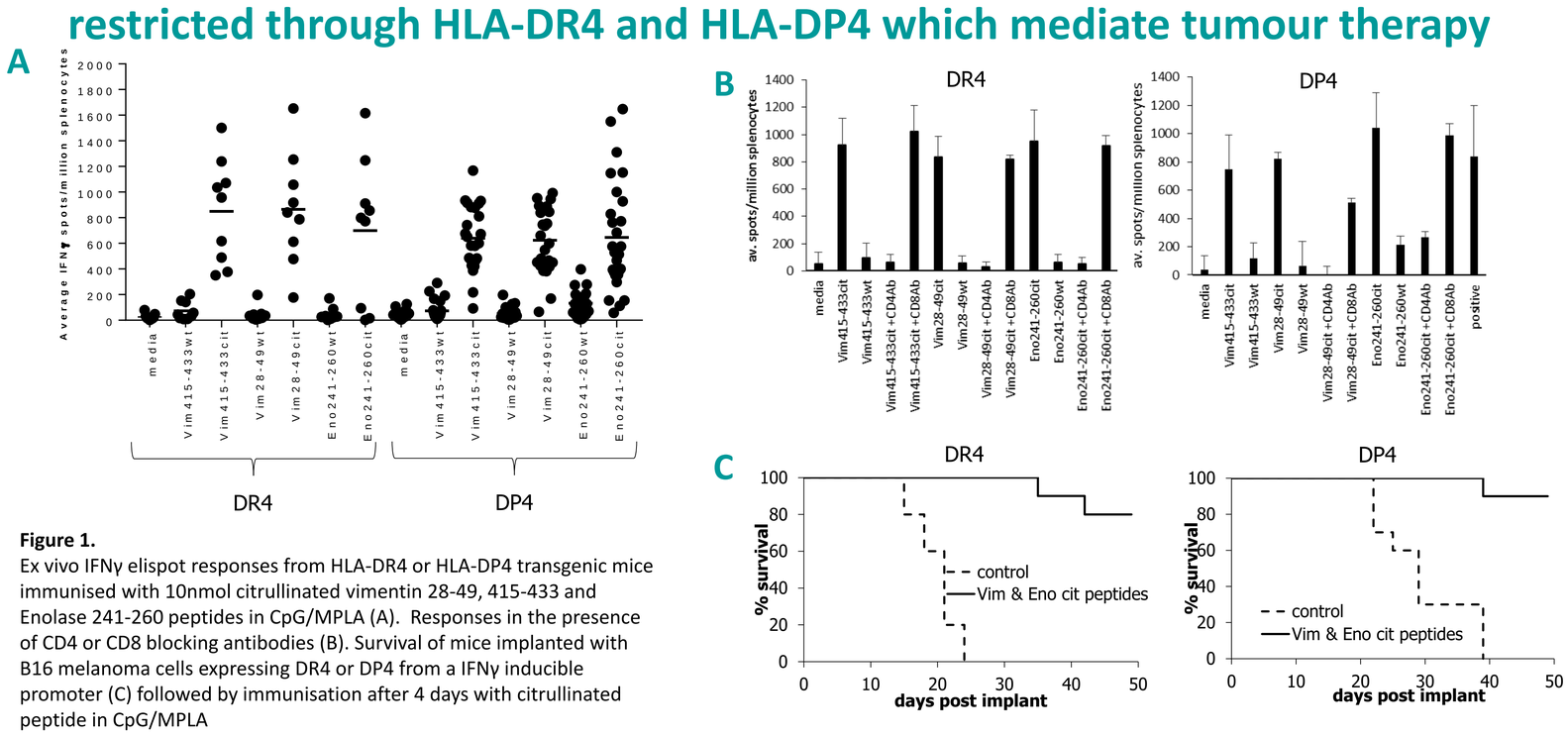


Figure 1.
Ex vivo IFNγ elispot responses from HLA-DR4 or HLA-DP4 transgenic mice immunised with 10nmol citrullinated vimentin 28-49, 415-433 and Enolase 241-260 peptides in CpG/MPLA (A). Responses in the presence of CD4 or CD8 blocking antibodies (B). Survival of mice implanted with B16 melanoma cells expressing DR4 or DP4 from a IFNγ inducible promoter (C) followed by immunisation after 4 days with citrullinated peptide in CpG/MPLA

Combination of citrullinated peptides with TLR1/2 agonist also induces Th1 responses.

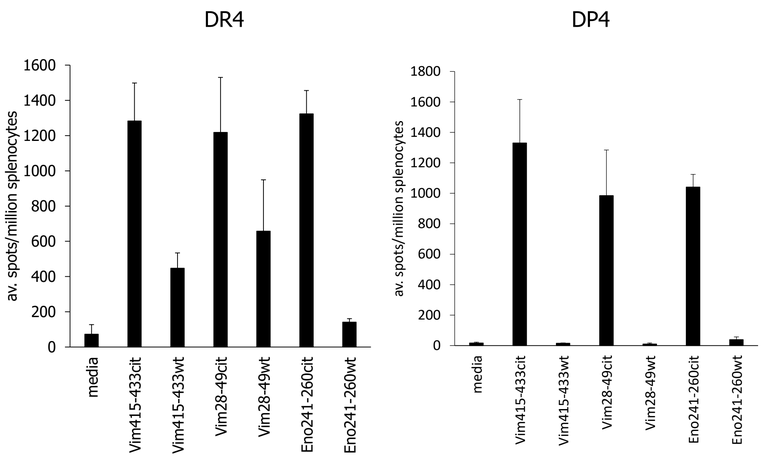
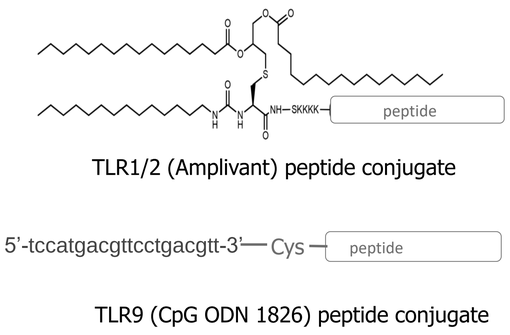


Figure 2.
Ex vivo IFNγ elispot responses from HLA-DR4 or HLA-DP4 transgenic mice immunised with 10nmol citrullinated vimentin 28-49, 415-433 or Enolase 241-260 peptides mixed with amplivant TLR1/2 ligand.

Conjugation of peptide to TLR agonist enhances immune responses at lower vaccine doses



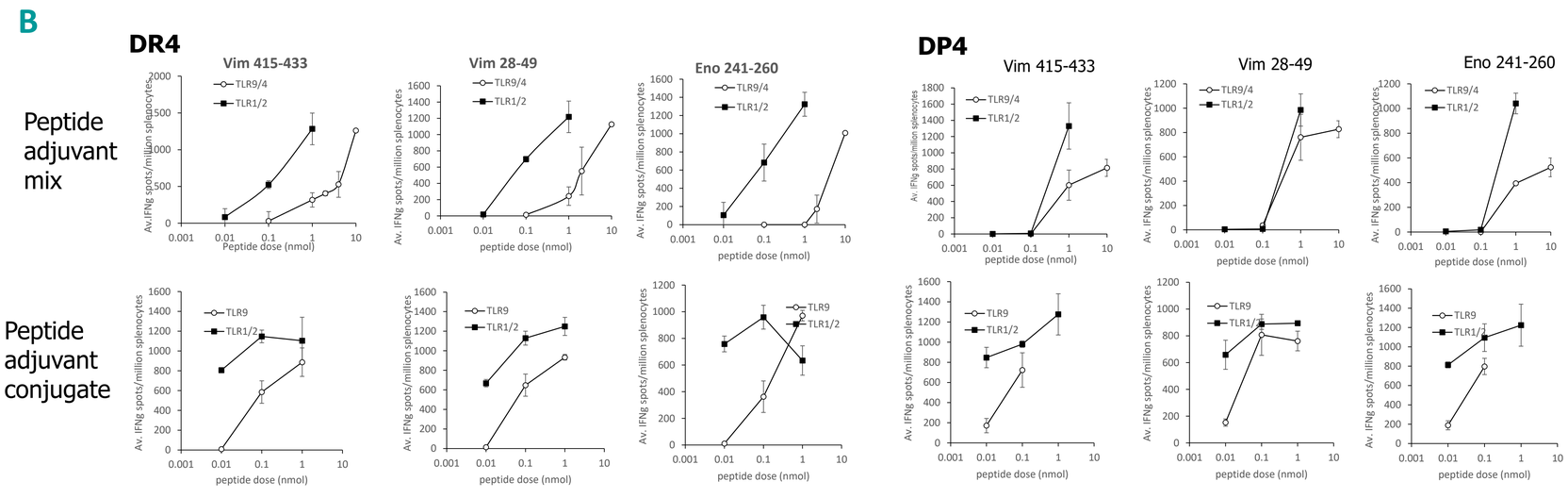


Figure 3.
Schematic representation of TLR agonist linked peptides (A). Ex vivo IFNγ elispot responses from HLA-DR4 or HLA-DP4 transgenic mice immunised with different doses of citrullinated vimentin 28-49, 415-433 and Enolase 241-260 peptides mixed with CpG/MPLA (TLR9/4) or TLR1/2 ligand or linked to CpG (TLR9) or TLR1/2 ligands (B).

Vaccination with low dose TLR1/2 or TLR9 agonist linked peptides mediate tumour therapy and protect against tumour rechallenge

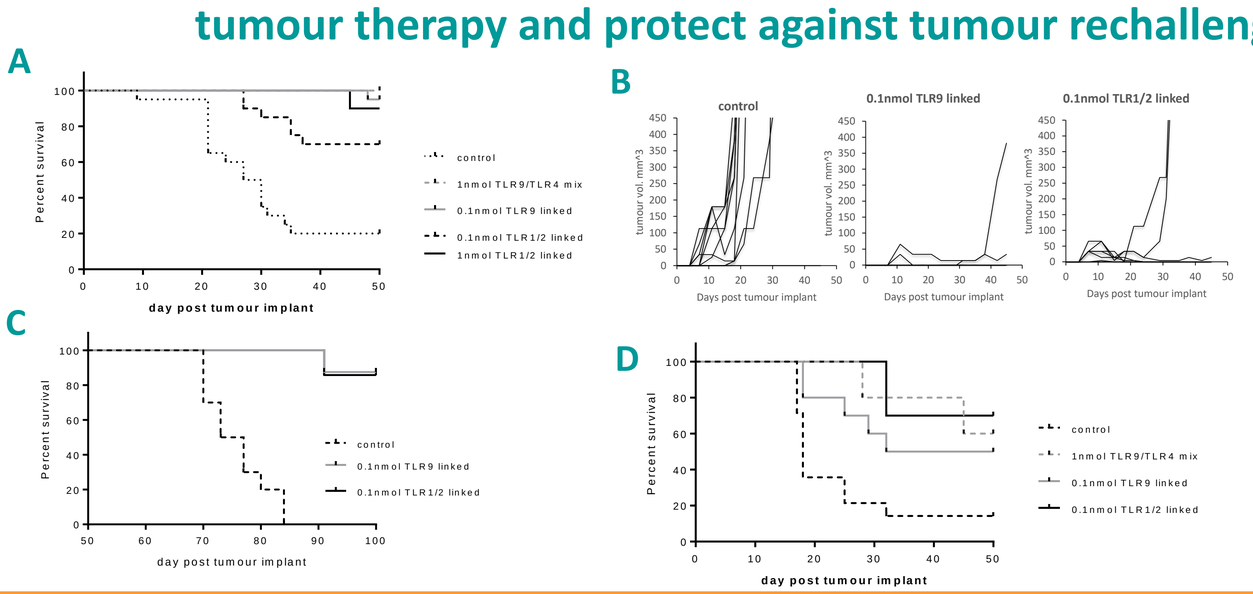


Figure 4.
Survival (A) and tumour growth (B) in DP4 mice implanted with B16 melanoma cells constitutively expressing DP4 followed by immunisation after 4 days with citrullinated peptide in mixed with or linked to TLR ligands. Survival following rechallenge at day 50 of tumour free DP4 mice (C). Survival in DR4 mice implanted with B16 melanoma cells expressing DR4 from a IFNγ inducible promoter (D).

CONCLUSIONS

- **Citrullinated peptides induce HLA-DR4 and HLA-DP4 restricted IFNγ responses**
- **IFNγ responses are CD4 mediated and provide efficient tumour therapy**
- **Responses can be stimulated in combination with TLR9 or TLR1/2 agonist**
- **Conjugation of peptide to TLR agonist enhances responses 10-100 fold**
- **Low dose TLR agonist linked peptides induce responses that show efficient tumour therapy and establish immunological memory to protect from tumour rechallenge.**
- **Peptide-TLR agonist conjugates enable better scaling of dose into human studies and should be considered for translation into the clinic**

References:

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